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The Incorporation of Radioactive Fatty Acids into the Phospholipids of Nerve-Cell-Body Membranes *in vivo*

EVIDENCE FOR HIGHLY LABELLED NEURONAL NUCLEI

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1. Nerve cell bodies were isolated in bulk from cerebral cortices of 15 day-old rabbits after intrathecal injections of [^{3}H]palmitate, [^{3}H]oleate or [^{3}H]arachidonate and [^{14}C]glycerol. 2. Nuclear, microsomal and two mitochondrial fractions were isolated from homogenates of the radioactively labelled nerve cell bodies by using differential and discontinuous-gradient centrifugation. 3. After 7.5 min *in vivo*, a high percentage (>80%) of the total ^{3}H-labelled fatty acid radioactivity was found in the membrane fractions of the nerve cell bodies, whereas after 60 min *in vivo* 50% of the total [^{14}C]glycerol radioactivity was found in the high-speed supernatant. 4. The specific radioactivities of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, and the radioactivity in neutral lipid and non-esterified fatty acid fractions were determined in the four subfractions, as were the distributions of several marker enzymes and nucleates. 5. With respect to ^{3}H-labelled fatty acid, the phospholipids of the nuclear fraction had the highest specific radioactivities of the four subfractions. However, for [^{14}C]glycerol labelling, generally the ^{14}C specific radioactivities for individual phospholipids were comparable in the four subfractions. This latter observation suggests transport of phospholipids synthesized *de novo* between membranes of the nerve cell body. 6. Double-labelling experiments demonstrated that individual phospholipids and the combined neutral lipids of the nuclear fraction had higher labelling ratios of ^{3}H-labelled fatty acid/[^{14}C]glycerol than did the corresponding lipids of the microsomal or mitochondrial fractions. 7. On the basis of the labelling results and the marker studies, it is proposed that it is indeed the nuclei of the nuclear fraction that have these lipids highly labelled with ^{3}H-labelled fatty acid, and the existence of nuclear acyl transferases that are responsible for this fatty acid incorporation is suggested.

Central nervous tissue has a high degree of cellular complexity, and subcellular fractions, such as mitochondria or microsomal fraction, prepared from whole brain or isolated brain areas, will inevitably be derived from a mixture of cells having often quite different functions (e.g. neurons and glia). Thus many investigations aimed at elucidating biochemical properties of brain nerve cells have been restricted to the use of synaptosomes and synaptosomal membranes. Although these particles are of great interest in studies of neurotransmitter turnover and the biosynthetic autonomy of the nerve ending, it is the neuronal perikaryon, with its richly developed endoplasmic reticulum, that is the principal centre for protein and lipid biosynthesis within the neuron (Jacobson, 1978). Over the past 12 years, a number of techniques have been developed to decrease the inherent cellular complexity in brain by the preliminary bulk isolation of cell classes such as neurons (chiefly the cell body), astrocytes and oligodendroglia (Rose, 1967; Blomstrand & Hamberger, 1969; Norton & Poduslo, 1970; Sellinger *et al.*, 1971; Poduslo & Norton, 1972; Nagata *et al.*, 1974). However, relatively few studies have been carried out on the composition and metabolic properties of subcellular fractions derived from these cells. Recently, we have analysed the composition of the lipids of several nerve-cell-body subfractions (Baker, 1979*b*), and now we present data concerning the metabolism *in vivo* of radioactive fatty acids and glycerol within the membranes of the neuronal perikaryon, with particular reference to the

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relatively high fatty acid labelling of lipids found in the neuronal nuclei.

Experimental

Materials

Monodur nylon mesh of pore sizes 335, 110 and 75 μm was provided by Tycan International, St. Catherines, Ont., Canada. Sigma Chemical Co (St. Louis, MO, U.S.A.) supplied the bovine serum albumin (Cohn fraction V), polyvinylpyrrolidone-40, cytochrome *c*, *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide, acetylthiocholine iodide, 5,5'-dithiobis-(2-nitrobenzoic acid), sodium pyruvate, NADH, DNA (calf thymus) and RNA (yeast). Bovine serum albumin (fatty acid-poor) was supplied by Miles Laboratories, Elkhart, IN, U.S.A. Accurate Chemical and Scientific Corp., Hicksville, NY, U.S.A., provided the metrizamide (Analytical grade). Digitonin was purchased from J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A., and Amido Black was from Allied Chemical Co., Morristown, NJ, U.S.A.

Lipid standards were purchased from Serdary Research Laboratories, London, Ont., Canada. Thin-layer plates (H) were obtained from Supelco Inc., Bellefonte, PA, U.S.A. New England Nuclear Corp., Lachine, Que., Canada, provided the scintillation solutions Aquasol and Econofluor and the following radiochemicals: [5,6,8,9,11,12,14,15-^{3}H]-arachidonic acid (sp. radioactivity 61 Ci/mmol); [U-^{14}C]glycerol (sp. radioactivity 100.1 Ci/mol); [9,10-^{3}H]oleic acid (sp. radioactivity 5.7 Ci/mmol); [9,10-^{3}H]palmitic acid (sp. radioactivity 12.1 Ci/mmol). Male New Zealand White rabbits (15 days of age) were purchased from Riemens Fur Ranches, St. Agatha, Ont., Canada.

Methods

Treatment of radioactive precursors. The radioactive fatty acids, supplied in organic solvents, were evaporated to dryness under a stream of N_2 and approximately neutralized in a small portion of 1 mM-NaOH. Bovine serum albumin (fatty acid-poor) dissolved in 5 mM-sodium phosphate buffer, pH 7.4, containing 0.9% NaCl was then added to give a molar ratio of fatty acid/albumin of 5 : 1. The mixtures were solubilized by gentle mixing for a few minutes (in the case of [^{3}H]palmitate, at 40°C). [^{14}C]Glycerol, supplied in aqueous 50% ethanol was taken to dryness under a stream of N_2 and redissolved in a small volume of 5 mM-sodium phosphate buffer, pH 7.4, in 0.9% NaCl.

Administration of radioactive precursor. The rabbit's scalp was treated with xylocaine and intrathecal injections of 25 μl were made over the cerebral cortical hemispheres. In double-label experiments, two injections were made per animal; the first contained approx. 14 μCi of [^{14}C]glycerol, and the second contained 15–30 μCi of ^{3}H-labelled fatty acid. The animals were divided into two groups, one receiving [^{14}C]glycerol for 30 min and ^{3}H-labelled fatty acid for 7.5 min, the second receiving [^{14}C]-glycerol for 60 min and ^{3}H-labelled fatty acid for 15 min before being killed.

Cellular fractionation and neuronal perikaryal subfractionation. After sacrifice, the cerebral cortices were removed and the method of Sellinger & Azcurra (1974) was followed for the isolation of neuronal perikarya. The nerve cell bodies were homogenized and primary subfractions were prepared by differential centrifugation as described previously (Baker, 1979*b*), with the exception that (*a*) to decrease microsomal contamination, the crude mitochondrial and lysosomal pellet (M + L) was sedimented at $1.7 \times 10^4 g_{max.}$ for 15 min, subsequently suspended in 0.25 M-sucrose and re-sedimented under the same conditions and (*b*) the pooled supernatants over fraction M + L were sedimented at $1.7 \times 10^4 g_{max.}$ for 15 min, and the resulting supernatant was sedimented at $1.05 \times 10^5 g_{av.}$ for 60 min to produce the microsomal pellet P and the high-speed supernatant S. The purified nuclear fraction (N_p) was prepared from the crude nuclear fraction N by density-gradient centrifugation as described previously (Baker, 1979*b*). The washed crude mitochondrial and lysosomal pellet M + L was suspended in 20% (w/v) metrizamide containing 50 μM-$CaCl_2$ and layered over a discontinuous step gradient consisting of 25%, 30% and 35% metrizamide in 50 μM-$CaCl_2$. After centrifugation in a swinging-bucket rotor (International Equipment Co., Needham Heights, MA, U.S.A.; rotor no. SB283) at $1.05 \times 10^5 g_{av.}$ in two sequential 30 min spins, two mitochondrial fractions were collected: fraction M-1 (30%/35% boundary), and fraction M-2 (pellet). Those fractions obtained as pellets (N_p, P and M-2) were resuspended in 0.25 M-sucrose.

Protein and enzyme assays. Protein was determined by the method of Lowry *et al.* (1951) or by the method of Schaffner & Weissmann (1973) for fractions suspended in metrizamide solution, with bovine serum albumin as standard in each case.

Total lactate dehydrogenase (EC 1.1.1.27) and cholinesterase (without inhibitor) activities were assayed as described by Whittaker & Barker (1972). Cytochrome *c* oxidase (EC 1.9.3.1) activity was measured by the method of Sottocasa *et al.* (1967). *N*-Acetyl-β-glucosaminidase was assayed as described by Sellinger *et al.* (1960). RNA was determined by the method of Fleck & Begg (1965) and DNA by the method of Burton (1965) with the terminal acetaldehyde addition noted by Giles & Myers (1965).

Lipid extraction, chromatography and analysis. Lipid extracts of subcellular fractions were prepared and purified by the method of Folch *et al.* (1957). Phospholipids, non-esterified fatty acids and neutral

lipids were separated by two-dimensional t.l.c. (Turner & Rouser, 1970). The lipids were detected by I_2 vapour. The gel areas were collected by aspiration into short Pasteur pipettes plugged with glass wool, and the phospholipids and neutral lipids were eluted with methanol or chloroform/methanol (2:1, v/v) respectively. Dried portions of phospholipid eluates or of the lipid extracts were submitted to acid hydrolysis and phosphate determination (Bartlett, 1959).

Aqueous radioactive samples were dissolved in 10ml of Aquasol. Samples of radioactive lipid in organic solvent were evaporated to dryness under a stream of N_2 and dissolved in 10ml of Econofluor. ^3H and ^{14}C radioactivities were determined simultaneously in a Nuclear Chicago mark II liquid-scintillation spectrometer with an external standard for calculating the efficiencies (^3H, 52–54%; ^{14}C, 59–66%). The specific radioactivities of labelled phospholipids were determined by taking portions for radioactivity and phosphate assays. Phospholipids, detected on chromatograms under u.v. light with 0.004% 2′,7′-dichlorofluorescein in methanol, were eluted, transmethylated and fatty acid methyl esters were extracted and analysed by argentation t.l.c., as described previously (Baker & Thompson, 1972).

Results

Distribution of marker enzymes, RNA and DNA

Table 1 shows the characterization of the four perikaryal subfractions used for lipid analysis, with respect to marker enzymes, DNA and RNA. The purified nuclear fraction (N_p) had the highest specific content of DNA and an RNA/DNA ratio of 0.45. The microsomal fraction P had the highest specific content of RNA and the highest specific activity of cholinesterase. The two mitochondrial fractions were enriched in cytochrome *c* oxidase activity, fraction M-1 having 3.9 times and fraction M-2 2.2 times the specific activity of the homogenate. All four perikaryal subfractions had low specific activities of *N*-acetyl-β-D-glucosaminidase compared with the homogenate. It was also noted that fractions N_p, M-1 and M-2 had less than 0.5% of the total activity of lactate dehydrogenase found in the homogenate, whereas approx. 6% of this activity was present in fraction P.

Phospholipid content of subcellular fractions

The nuclear fraction N_p had a very low specific phospholipid content [0.106 ± 0.02 ($n = 10$) μmol of P/mg of protein (mean ± S.D.)], and this was the lowest of the four perikaryal subfractions under study, e.g. microsomal fraction P had a phospholipid content of 0.488 ± 0.06 ($n = 10$) μmol of P/mg of protein (mean ± S.D.). Table 2 shows the percentage distributions of phosphate among the phospholipids of the four perikaryal subfractions. Phosphatidylcholine and phosphatidylethanolamine made up the bulk of the phospholipid in each case, but with respect to the minor phospholipids, the nuclear fraction N_p and the two mitochondrial fractions had phosphatidylinositol at approx. 3-fold higher values than phosphatidylserine. This was in marked contrast with the microsomal fraction P, which had approximately equal contributions from these two phospholipids. The two mitochondrial

Table 1. *Marker enzyme, DNA and RNA concentrations in homogenates of neuronal perikarya and derived subfractions*
The methods of enzyme and nucleate assay are given in the text. Neuronal perikarya were isolated in bulk from rabbit cerebral cortex and perikaryal subfractions were isolated from homogenates of the cell bodies by differential and discontinuous-gradient centrifugation. The four subcellular fractions studied were: N_p (purified nuclei); P (microsomal fraction); M-1 (mitochondria-1); M-2 (mitochondria-2). The spectrophotometric assays used cuvettes with a 1cm light path. The results are means ± S.D. for three or more independent determinations. The protein content of the nerve-cell-body homogenate was based on the sum of the quantities of protein found in the primary membrane subfractions (N, M + L, M + L wash, P). The recovery values for the nucleates do not include the high-speed supernatant fraction S. The units used are: cytochrome *c* oxidase, ΔA_{550}/min; cholinesterase, ΔA_{412}/min; *N*-acetylglucosaminidase, ΔA_{410}/min.

	Activity (units/mg of protein)			Concn. (μg/mg of protein)	
Fraction	Cytochrome *c* oxidase	Cholin-esterase	*N*-Acetyl-glucosaminidase	RNA	DNA
Homogenate (cell bodies)	5.4 ± 1.1	0.9 ± 0.1	19.9 ± 1.3	349 ± 36	176 ± 23
N_p	0.15	0.12	1.0	99.5	223
P	0.28	1.7	5.9	469	7.8
M-1	21.1	0.7	5.1	119	5.3
M-2	11.8	1.0	3.6	161	42.2
Recovery (%)	82	79	86	65	68

Table 2. *Phospholipid compositions of neuronal perikaryal subfractions*

By using differential and discontinuous-gradient centrifugation, subcellular fractions N_p (nuclei), P (microsomal), M-1 and M-2 (mitochondria-1 and -2) were prepared from homogenates of neuronal perikarya. Phospholipids were extracted from the subfractions and were separated by two-dimensional t.l.c. Phospholipids were quantified by the phosphate assay. The results are means ± S.D. and are expressed as percentages of the total phosphate in the five phospholipids under study. The numbers of independent determinations are given in parentheses.

	Phospholipid (% of total phospholipid phosphate)			
	N_p (9)	P (8)	M-1 (10)	M-2 (10)
Phosphatidylcholine	59.1 ± 4.3	54.1 ± 3.3	51.2 ± 2.9	48.4 ± 1.5
Phosphatidylethanolamine	26.5 ± 2.3	29.7 ± 2.3	30.8 ± 1.4	32.9 ± 1.2
Phosphatidylinositol	10.5 ± 0.8	7.0 ± 0.7	8.7 ± 1.1	8.2 ± 1.0
Phosphatidylserine	3.9 ± 0.8	9.2 ± 0.9	3.0 ± 0.7	2.5 ± 0.4
Cardiolipin	—	—	6.4 ± 0.4	8.0 ± 0.5

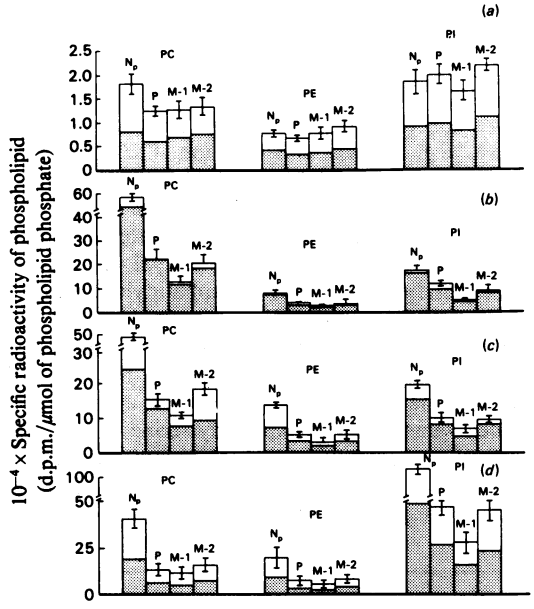

Fig. 1. *Histogram of specific radioactivities of phospholipids of fractions N_p, P, M-1 and M-2 derived from nerve cell bodies of rabbit cerebral cortex*

The specific radioactivities of the phospholipids expressed per μmol of phospholipid phosphate are presented as means ± S.D. for five ([^{14}C]glycerol) or three ([^{3}H]palmitate) independent determinations (for [^{3}H]oleate and [^{3}H]arachidonate, averages ± ranges). The stippled blocks represent the specific radioactivities after 7.5 min for ^{3}H-labelled fatty acid or 30 min for [^{14}C]glycerol *in vivo*, and the upper limiting bars represent the values after 15 min for ^{3}H-labelled fatty acid or 60 min for [^{14}C]glycerol *in vivo*. Rabbits were injected intrathecally with approx. 14 μCi of [^{14}C]glycerol and/or 15–30 μCi of ^{3}H-labelled fatty acid. Animals were killed, cerebral cortices were removed and nerve cell bodies were prepared in bulk. A pool of four animals was used for each time point. From homogenates of the nerve cell bodies four subcellular fractions were isolated, which were analysed for phospholipid. Abbreviations used: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol. The radioactive precursors used were: (*a*), [^{14}C]glycerol; (*b*), [^{3}H]palmitate; (*c*), [^{3}H]oleate; (*d*), [^{3}H]-arachidonate.

fractions showed the characteristic mitochondrial phospholipid cardiolipin at values of 6.4 and 8.0%, which were comparable with values for cardiolipin of mitochondria directly isolated from rabbit cerebral cortex (Hamberger & Svennerholm, 1971).

Radioactive labelling of subcellular fractions

After 7.5 min *in vivo*, more than 80% of the ^{3}H-labelled fatty acid radioactivity in the nerve-cell-body homogenate was found in the primary membrane fractions (N, M + L and P), whereas approx. 50% of the perikaryal [^{14}C]glycerol radioactivity was found in the high-speed supernatant S after 60 min *in vivo*. The amounts of radioactivity in lipid extracts of the nuclear fraction N_p, labelled with ^{3}H-labelled fatty acids, were 70–90% of the total ^{3}H radioactivity found in extracts of the microsomal fraction P. In contrast, values for [^{14}C]glycerol radioactivity in the nuclear lipid extract were less than one-third of the corresponding microsomal values. There was no ^{14}C radioactivity demonstrable in the non-esterified fatty acid fraction of any of the extracts. Argentation t.l.c. of fatty acid methyl esters derived from lipid extracts after 15 min *in vivo* indicated less than a 4% change in the degree of unsaturation of the injected radioactive oleate and arachidonate, and 6% of the injected radioactive palmitate was associated with monoenoic fatty acids.

Fig. 1 shows the specific radioactivities of phospholipids (d.p.m./μmol of phosphate) in the nuclear (N_p), microsomal (P) and mitochondrial fractions (M-1 and M-2) after 7.5 or 15 min of labelling by ^{3}H-labelled fatty acid or 30 or 60 min of

labelling by [^{14}C]glycerol *in vivo*. The specific radioactivities of the individual phospholipids (phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) of the nuclear fraction labelled with ^{3}H-labelled fatty acids were two to three times the corresponding values for individual phospholipids in the microsomal fraction. Comparing the two time points, this fatty acid labelling differential was generally maintained or increased with time. By contrast, the two mitochondrial fractions M-1 and M-2 had phospholipids with ^{3}H specific radioactivities that were respectively less than or comparable with the corresponding values in the microsomal fraction. The specific radioactivities of [^{14}C]-glycerol in the phospholipids of the four perikaryal subfractions showed a different pattern. With the exception of higher ^{14}C specific radioactivities in phosphatidylcholine of the nuclear fraction after 60min *in vivo* ($P < 0.05$, comparing fractions N_p and P by Student's *t* test), generally the ^{14}C specific radioactivities in individual phospholipids were comparable among the four subfractions.

With the exception of the nuclear fraction, where ^{14}C specific radioactivities for phosphatidylcholine and phosphatidylinositol were quite similar, the order of specific radioactivities for [^{14}C]glycerol labelling was: phosphatidylinositol > phosphatidylcholine > phosphatidylethanolamine. This was also the order for phospholipids labelled with [^{3}H]-arachidonate in each of the subfractions. However, labelling by [^{3}H]palmitate or [^{3}H]oleate showed the specific radioactivity order: phosphatidylcholine > phosphatidylinositol > phosphatidylethanolamine. Phosphatidylserine and cardiolipin (in fractions M-1 and M-2) had specific radioactivities that were generally much lower than the corresponding values for phosphatidylethanolamine. One exception to this was the closeness of [^{3}H]-arachidonate specific radioactivities in phosphatidylserine and phosphatidylethanolamine.

Generally, there were increases with time in the specific radioactivities of each of the phospholipids in the subfractions, particularly for radioactive labelling with [^{14}C]glycerol, [^{3}H]oleate and [^{3}H]-arachidonate. For [^{3}H]palmitate, however, the specific radioactivities at the two time points were often quite close, suggesting that a maximum had been approximated by the early time point (7.5min).

Table 3 presents the ratios of specific radioactivities of ^{3}H-labelled fatty acid/[^{14}C]glycerol for the phospholipids of the four perikaryal subfractions derived from double-label experiments. In each case, at each time, it was the nuclear fraction N_p that had the highest double-label ratio. For the three phospholipids, the nuclear ratios were 1.5–2.6 times the corresponding microsomal ratios. For the nuclear neutral lipids, this differential was increased to 2.4–3.9 times the microsomal neutral lipid ratios.

Table 3. *Ratios of labelling of incorporated ^{3}H-labelled fatty acid/[^{14}C]glycerol in phospholipids and neutral lipid of subcellular fractions of neuronal perikarya*

Rabbits were injected intrathecally at different times with ^{3}H-labelled fatty acid and [^{14}C]glycerol, and were sacrificed after (*a*) 7.5min for fatty acid and 30min for glycerol or (*b*) 15min for fatty acid and 60min for glycerol. Nerve cell bodies were isolated from the cerebral cortices and subcellular fractionation yielded fractions N_p (purified nuclei), P (microsomal), M-1 (mitochondria-1) and M-2 (mitochondria-2). These fractions were analysed for phospholipid. Results are averages of two independent experiments for each fatty acid. For comparisons between fatty acids, ratios were determined on the basis of a ^{3}H/^{14}C radioactivity (d.p.m.) ratio of unity for the injected radioactive precursors.

		^{3}H/^{14}C Ratio							
		Phosphatidylcholine		Phosphatidylethanolamine		Phosphatidylinositol		Neutral lipid	
Precursors	Fraction	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
[^{3}H]Palmitate, [^{14}C]glycerol	N_p	46.5	24.3	14.1	7.6	16.6	8.1	51.4	29.0
	P	31.5	14.9	7.1	4.0	8.4	4.2	19.3	10.4
	M-1	15.9	9.2	3.5	1.9	4.8	2.5	9.7	7.8
	M-2	22.3	13.4	5.2	2.5	6.9	3.0	17.8	9.4
[^{3}H]Oleate, [^{14}C]glycerol	N_p	8.2	8.2	5.3	5.9	5.6	4.2	7.6	8.7
	P	5.1	4.9	3.0	3.0	2.6	2.4	2.3	2.8
	M-1	3.2	3.3	1.5	1.4	1.8	1.4	2.2	2.3
	M-2	4.2	4.7	2.4	2.4	2.6	2.0	3.3	3.6
[^{3}H]Arachidonate, [^{14}C]glycerol	N_p	18.9	26.7	24.6	26.9	63.9	60.1	11.1	15.5
	P	11.6	10.2	12.2	11.3	33.2	24.3	4.6	4.9
	M-1	9.1	8.3	6.4	6.6	20.7	16.9	2.9	2.3
	M-2	13.2	11.1	9.7	7.7	32.5	20.6	5.0	5.6

The two mitochondrial fractions showed double-label ratios in their lipids, which were usually less than or close to the corresponding microsomal values.

In Table 4 are given the percentage distributions of radioactivity among the lipids of the nuclear (N_p) and microsomal (P) fractions after 7.5 min of ^{3}H-labelled fatty acid or 60 min of [^{14}C]glycerol labelling. Phosphatidylcholine in each case accounted for the bulk of the radioactivity (48–70% of the total). For labelling by [^{14}C]glycerol, [^{3}H]palmitate and [^{3}H]oleate, the neutral lipid fraction made the second highest contribution to the total (13–29%), whereas for [^{3}H]arachidonate labelling, it was phosphatidylinositol that made the second highest contribution (23–25%). Non-esterified fatty acids represented a relatively small proportion of the ^{3}H labelling in each case (<5%), although this fraction did make a larger contribution to the total nuclear lipid (3–4%) than to the total microsomal lipid (0.2–0.8%) radioactivity. Similarly, for labelling by ^{3}H-labelled fatty acid, the nuclear neutral lipids were more prominent than were the corresponding microsomal neutral lipids. This was in contrast with the contributions made by neutral lipids labelled with [^{14}C]glycerol, where the values for the nuclear and microsomal fractions were the same.

Discussion

We have made use of the cellular isolation step in these studies because it allows the analysis of subcellular membranes derived from one cell class and makes comparisons between different organelles or membrane fractions more meaningful. It also eliminates the potential for contamination by myelin fragments, synaptosomes, erythrocytes and tissue debris found in homogenates of whole-brain tissue. The purity of the neuronal perikarya was checked by phase-contrast microscopy and by the use of certain enzyme markers as noted previously (Baker, 1979*a*,*b*). The method of homogenizing the nerve cell bodies gives a reasonably complete disruption of the perikarya to their component organelles and membranes, and thus removes the possibility of contamination of the crude nuclear fraction by partially broken cell bodies (Baker, 1979*b*). The sedimentation of the neuronal nuclei through heavy sucrose decreased the already low amounts of light-membrane contamination found in the crude nuclear fraction N, as judged by several marker enzymes (Table 1). The purified nuclear fraction, N_p, had amounts of DNA and RNA/DNA ratios that were comparable with values for isolated neuronal nuclei given in the literature (Giuditta *et al.*, 1972; Thompson, 1973). The microsomal fraction P had the highest specific content of RNA of the four perikaryal subfractions. This value was similar to specific contents of RNA found in microsomal fractions derived from nerve cell bodies isolated from 18 day-old rat brain (Johnson & Sellinger, 1971). These microsomal RNA values are at least four to five times the specific contents of RNA found in microsomal fractions derived from rat whole brain (Morgan *et al.*, 1971; Tamai *et al.*, 1974) and this may indicate a far higher percentage content of rough endoplasmic reticulum in the microsomal fractions derived from isolated nerve cell bodies. Of the four fractions studied, the microsomal fraction P had also the highest specific activity of cholinesterase, an enzyme found in plasma membrane and endoplasmic reticulum of cholinergic cells. A similar finding was also made for the more specific endoplasmic reticulum marker, NADPH–cytochrome *c* reductase, in fraction P. The two mito-

Table 4. *Distribution of radioactivity among lipids of fractions N_p and P*

Lipid extracts of fractions N_p and P were prepared and the four major phospholipids, neutral lipid and non-esterified fatty acid were resolved by two-dimensional t.l.c. The radioactivity (d.p.m.) in each was determined and expressed as a percentage of the total. The results are means ± S.D. or averages ± range. The numbers of independent determinations are given in parentheses and the times *in vivo* for each radioactive precursor were: [^{14}C]glycerol, 60 min; ^{3}H-labelled fatty acid, 7.5 min.

	Distribution of radioactivity (%)							
	[^{14}C]Glycerol (5)		[^{3}H]Palmitate (3)		[^{3}H]Oleate (2)		[^{3}H]Arachidonate (2)	
Lipid	N_p	P	N_p	P	N_p	P	N_p	P
Phosphatidylcholine	60.8 ± 3.1	54.5 ± 0.8	61.6	67.5	53.8	69.5	48.7	47.8
Phosphatidylethanolamine	10.6 ± 1.2	15.2 ± 1.1	4.7	5.3	6.9	10.5	10.4	14.0
Phosphatidylinositol	8.9 ± 0.8	9.9 ± 1.4	3.8	4.7	6.7	5.7	22.7	25.2
Phosphatidylserine	0.4 ± 0.3	1.2 ± 0.2	0.3	0.9	0.4	0.8	0.8	4.0
Neutral lipids	19.3 ± 3.5	19.3 ± 1.7	25.6	20.9	29.0	12.6	12.9	8.9
Non-esterified fatty acids	—	—	4.2	0.7	3.2	0.8	4.3	0.2

chondrial fractions M-1 and M-2 together accounted for approx. 80% of the cytochrome *c* oxidase activity found in fraction M + L. This enzyme was more enriched in fraction M-1, yet the two fractions did have similar amounts of the mitochondrial phospholipid cardiolipin. On the basis of phospholipid phosphate concentrations, the nuclear and mitochondrial fractions had phosphatidylinositol/phosphatidylserine ratios of 2.7–3.3, whereas the corresponding value for the microsomal fraction was 0.8. This was comparable with values for subfractions of neuronal perikarya prepared from adult rabbit cerebral cortex (Baker, 1979*b*).

The nuclear phospholipid content (per mg of protein) was approx. 20% of the corresponding value for the microsomal fraction. In a recent review, the majority of techniques listed for the isolation of liver nuclear envelopes yielded preparations with specific phospholipid contents that were in the range 0.4–0.5 μmol of phosphate/mg of protein (Harris, 1978), compared with values for whole nuclei from rat liver of 0.052 (Gurr *et al.*, 1963) and 0.089 (Chauveau *et al.*, 1956). The bulk of the nuclear protein is probably found in chromatin, leaving a small contribution to be made by the proteins in the nuclear membranes. This dilution factor should be taken into consideration when evaluating in whole nuclear fractions the specific activities of enzymes found in the nuclear envelope.

The radioactivity data indicated a very rapid uptake of ^{3}H-labelled fatty acid into complex lipids and into the membranes of the nerve cell bodies. In contrast, [^{14}C]glycerol radioactivity, after 60 min *in vivo*, was still found in substantial quantities (50% of total) in the high-speed supernatant. Our interest in nuclei came from the observation that the nuclear fraction N_p, although of much lower phospholipid content, nonetheless had amounts of ^{3}H-labelled fatty radioactivity that approached those of the microsomal fraction P. The specific ^{3}H radioactivities in individual phospholipids of the nuclear fraction were in fact two to three times or more the corresponding values for the microsomal or mitochondrial phospholipids. This large fatty acid labelling differential and the low amounts of other membrane contaminants in fraction N_p strongly suggest that these highly labelled phospholipids in this fraction are indeed nuclear phospholipids. Analyses of subfractions isolated from neuronal perikarya of 8 day- and 22 day-old rabbit cerebral cortex, labelled with [^{3}H]oleate *in vivo*, have also shown these high nuclear phospholipid specific radioactivities.

In contrast, the [^{14}C]glycerol labelling studies indicated that the four perikaryal subfractions have individual phospholipids that generally have much closer ^{14}C specific radioactivity values, with the exception of nuclear phosphatidylcholine at 60 min. Somewhat similar results were reported by Miller & Dawson (1972) in [^{32}P]P_i labelling studies of guinea-pig brain *in vivo*, where mitochondrial phospholipid specific radioactivities were often close to microsomal values. Assuming that the microsomal fraction (representing the endoplasmic reticulum) is the chief site for complex phospholipid biosynthesis (Dawson, 1973), the similarity in phospholipid specific radioactivities in different subcellular fractions labelled by [^{14}C]glycerol implies a rapid transport of newly synthesized phospholipids from the endoplasmic reticulum to other membranes of the nerve cell body. This exchange, mediated by phospholipid-exchange proteins, has been demonstrated *in vitro* for liver and brain subfractions (Wirtz & Zilversmit, 1968; McMurray & Dawson, 1969; Miller & Dawson, 1972).

Double-labelling experiments were used in these studies to explore the possibilities of lipid acylation reactions occurring in membranes other than the endoplasmic reticulum, e.g. lysophospholipid acylation (independent of synthesis *de novo*) or the acylation of neutral lipids. Assuming that phospholipids newly synthesized *de novo* would be largely imported from the endoplasmic reticulum, independent acylation reactions at a second membrane site would be shown by higher fatty acid/glycerol lipid labelling ratios. As the nuclear phospholipids and neutral lipids had 1.5–4-fold higher double-label ratios than those of the microsomal lipids for three different fatty acids, it is reasonable to suggest the existence of neuronal nuclear acylation reactions. The fatty acid radioactivity in the neutral lipid fraction has not been localized in a specific acyl ester, but on the basis of studies with whole brain (MacDonald *et al.*, 1975; Sun & Yau, 1976), and with liver nuclear neutral lipid (Kleinig, 1970), diacylglycerol, triacylglycerol and cholesteryl ester are likely candidates for the labelling by ^{3}H-labelled fatty acid. As these lipids occur in small quantities in brain, it is likely that they have very high ^{3}H specific radioactivities (MacDonald *et al.*, 1975). A previous study with liver subcellular fractions did indicate that specific activities for acyl-CoA–1,2-diacyl-*sn*-glycerol acyltransferase and acyl-CoA–1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in whole nuclear fractions were 70–80% of the corresponding values in smooth and rough microsomal fractions when these activities were expressed per μmol of phospholipid phosphate in the subfractions (Van Golde *et al.*, 1971). The latter paper also noted specific activities for nuclear choline phosphokinase, which were more than 40% of the microsomal activities when expressed in this manner. Assuming these enzymes are found in the nuclear envelope, this is a preferable way of expressing nuclear specific activities. Stadler & Franke (1973) have shown that hen erythrocyte

nuclei *in vitro* can incorporate radioactive palmitate and oleate into phospholipids of the nuclear membranes. The enzyme that catalyses the formation of CDP-diacylglycerol from phosphatidate has also been shown to be present in neuronal nuclei and specifically in the nuclear envelope (Thompson, 1975, 1977).

In the present studies, the [^{14}C]glycerol specific radioactivities in phosphatidylinositol were equal to or 20–70% higher than the specific radioactivities in phosphatidylcholine in any one subfraction. Much higher ratios of [^{14}C]glycerol specific radioactivities for phosphatidylinositol/phosphatidylcholine were found in labelling studies of whole brain *in vivo* [2.5 after 60min (Baker & Thompson, 1972); 5.1 after 30min (Braünig & Gercken, 1976)], or for incorporation studies with synaptosomes *in vitro* [7.5 after 15min (Baker *et al.*, 1976); 9.5 after 30min (Lunt & Lapetina, 1970)]. This serves to emphasize possible differences in the relative rates of synthesis *de novo* of phosphatidylinositol at the nerve ending, and possibly in other cells when compared with neuronal perikarya.

After 7.5min *in vivo*, the ^{3}H-labelled fatty acids had been metabolized very quickly (in agreement with previous reports of fatty acid metabolism in brain, described by Sun & Horrocks, 1971). Nonetheless, the specific radioactivities of phospholipids labelled with [^{3}H]oleate or [^{3}H]arachidonate did increase substantially by the second time point (15min). The source of the additional fatty acid may be the neutral lipid fraction, and it has been postulated that less polar lipids may serve as a holding reservoir for fatty acids entering the brain (Dwyer & Bernsohn, 1979). The high contributions to the total lipid radioactivity made by phosphatidylcholine for [^{3}H]oleate and [^{3}H]palmitate labelling and by phosphatidylinositol for [^{3}H]-arachidonate labelling reflect the fatty acid compositions of these two phospholipids (Baker, 1979*a*). In fact, when the ^{3}H specific radioactivities (Fig. 1) are expressed per μmol of fatty acid in each phospholipid, it is phosphatidylinositol, labelled with [^{3}H]palmitate or [^{3}H]oleate, and phosphatidylcholine, labelled with [^{3}H]arachidonate, that are the most highly labelled phospholipids in the nuclear and microsomal fractions.

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